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The Effect of Air Humidity on the Viability of Microorganisms in an Aerosol

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The liberation of air from viable microorganisms is a complex process which is dependent on various factors. In a small closed space it depends both on the process of bacterial decay in the conditions of an air medium and on the process of bacterial sedimentation. Sedimentation is a purely colloidal process and therefore its speed depends mainly on the sizes of the particles and the droplets of the bacterial aerosol, and the presence, though it is very insignificant, of air currents.

The stability of various microorganisms in the air fluctuates within wide limits. Thus for example, Staph. aureus, C. diphthereiae and Bac. subtilis maintain their viability considerably longer in a drop phase aerosol than Haemophilus influenzae, Bact. coli, Bact. prodiguosum (Wells. W., Wells, M., 1936; Thomas, 1955 and others). The temperature and humidity of the surrounding air exert a tremendous influence on the viability of bacteria in an aerosol (Wells and Zappassadi, 1942; Yarnykh, 1957; De Ome, 1944; Dimmick, 1960; Dunklin and Puck, 1948; Griffin and others, 1956; Webb, 1959, 1960).

The aim of the present work was the study of the effect of air humidity on microorganisms in a drop phase bacterial aerosol. In the role of experimental model they used bacteria which is characteristic for the air of closed premises - Staph. albus and Sarcina lutea, which were isolated from the air in the laboratory. In another group they included microorganisms that were not characteristic for the microflora of air, namely Bact. coli and Bact. prodigiosum which were isolated from water.

METHODS

The experiments with bacterial aerosols were carried out in an experimental chamber with a capacity of 250 liters, in which suspensions of bacteria were dispersed. Cuvettes with dry calcium chloride were brought in to lower the air humidity in the chamber, and for increasing the humidity steam was introduced into the chamber. In that way they succeeded in creating a relative humidity within the limits of 12 to 98%. Since it is extremely difficult to reproduce an identical air humidity; the experiments were carried out within determined spans of humidity: lower than 20%, from 30 to 40%, from 50 to 60%, from

70 to 80%, and higher than 90%. Along with this, the temperature variation was within the limits of from 18.5 to 21.0°.

Daily cultures of Staph. albus, Bact. prodigiosum, Bact. coli and Sarcina lutea in agar slants were washed off with a physiological solution and diluted to a concentration of 300 million bacterial cells in 1 ml. In each individual experiment, a 0.12 ml bacterial suspension was dispersed.

Air samples were taken by the method of sedimentation in Petri dishes immediately after the creation of the aerosol, after 10 minutes, and after 20 minutes; each dish was exposed for 10 minutes. In that manner, each individual experiment continued for 30 minutes, after which the air in the chamber was disinfected with the help of bactericidal ultra-violet radiation (BUV-15 lamp).

In setting up the experiments with Bact. coli, rosolic agar and Endo medium were used, for Bact. prodigiosum - glucose-starch agar, and for Staph. albus and Sarcina lutea - meat-peptone agar. Dishes with a culture of Bact. coli were cultivated for 24 hours, with Staph. albus and Sarcina lutea - two days at a temperature of 37°, and Bact. prodigiosum - 24 hours at a temperature of 28°.

In all, 18 series of basic experiments were conducted. This number doesn't take into account preparatory experiments in which methods were worked out and preliminary results obtained. Each basic series of experiments consisted of 8 - 10 individual experiments which were carried out in one day with a suspension of one culture at a different air humidity, meaning that there were two experiments for each span of humidity.

Besides this, in order to determine the number of viable microorganisms for a specific volume of air, series of experiments were conducted with Staph. albus and Bact. prodigiosum. Air cultures of 1, 5, and 10 liters were produced on No. 4 membrane filters by means of drawing the air through them 10 minutes after the creation of the bacterial aerosol. Immediately after the selection of air samples, the membrane filters are placed in the appropriate nutritive media.

RESULTS OF THE INVESTIGATIONS

During the conduct of the experiments, two types of changes in the concentration of viable bacteria in the air at various humidities were obtained dependent on the species they belonged to. One type of change was obtained in the gram-positive cocci Staph. albus and Sarcina lutea, the other type was produced by the gram-negative bacilli, Bact. coli and Bact. prodigiosum.

Changes in the air humidity didn't exert a significant influence on the amount of drop settling of the bacterial aerosol of Staph. albus and Sarcina lutea. As is apparent from the table, approximately the same number of drops which contain viable Staphylococci settle during the various humidities. Only a small reduction is observed in the number of staphylococci during the low rates of humidity and a greater reduction in their number when the humidity is higher than 90%. However, the difference is so small that on a semi-logarithmic graph the curves for the settling of staphylococci at various humidities practically run together. Analogous results were also obtained in other experiments with Staph. albus and also in experiments with Sarcina lutea. In individual investigations several large variations were observed in the number of colonies growing on the dishes, which are more often observed in experiments with Sarcina.

The facts expressed above are demonstrated by the exceedingly rapid evaporation of the layer of water surrounding the bacteria during low air humidity, as a result of which the size of the drops rapidly decreases and their sedimentation velocity is sharply curtailed. On the contrary, during a high humidity, the size of the drops will hardly get smaller and may even become somewhat larger due to moisture condensation. Under these conditions the bacterial drops settle more rapidly than during low and moderate levels of humidity which leads to a decrease in the concentration of the bacterial aerosol. However this doesn't exclude the possibility of somewhat of a decrease in the number of bacteria in the air due to an acceleration in their rate of decay at high humidities.

Thus it can be assumed that the content of Staph. albus and Sarcina lutea in the drop phase of a bacterial aerosol is subject to the influence mainly of colloidal factors which change depending on the air humidity. A decrease in the concentration of bacteria in the air takes place basically due to the settling of bacterial drops. Then the process of decay for these bacteria has a secondary significance.

On the other hand the relative air humidity exerts a great influence on the viability of Bact. coli and Bact. prodigiosum under aerosol conditions. The data presented in figures 1 and 2 show that in a relative humidity lower than 40% these microorganisms are completely destroyed in the course of 30 minutes after the bacterial suspension has been dispersed. If, during the first 10 minutes of the experiment, tens and hundreds of colonies are growing on the Petri dish, then after 10 and 20 minutes only single colonies can be determined. In a high air humidity the amount of viable bacteria determined is much greater even though an equal amount of bacterial suspension was dispersed in the chamber. In a relative humidity of from 50 to 60%, the survival rate of Bact. coli increased considerably more than that of Bact. prodigiosum. A very large amount of both microorganisms was determined

in a relative humidity greater than 70%, they exceeded by tens and sometimes by hundreds the number of bacteria determined at low levels of humidity. In a number of experiments in a humidity greater than 90%, less viable bacteria was determined than in humidity within the limits of 70 - 80%, whereas in other experiments opposite results were obtained.

For determining the number of viable Bact. prodigiosum in a certain volume of air at various humidities, air samples were taken on membrane filters (fig. 3). The following results were obtained. In 1, 5, and 19 liter cultures of air at a relative humidity of 37%, there grew correspondingly 1 - 2, 6 - 9, and 11 - 12 colonies of Bact. prodigiosum. In a humidity of 75% all the cultures yielded a solid growth on membrane filters. A 1 liter air culture at a relative humidity of 93% yielded a growth of 146 - 200 colonies, a 5 liter culture - more than 500 - 600 colonies, and a 10 liter culture - a solid growth. It wasn't considered feasible to obtain more precise data in as much as calculation of more than 100 - 150 colonies on a membrane filter is extremely difficult. On figure 3 it is apparent that part of the Bact. prodigiosum colonies have lost the ability for pigment formation. In [illegible]ations of culture suspension there was also observed the formation of rose-colored and colorless variants. We weren't able to establish the dependence between the number of rose and apigmentary variants and the relative air humidity in which the aerosol of Bact. prodigiosum was produced.

DISCUSSION OF RESULTS

In conformance with the data of a number of authors and our experiments, the stability of bacteria in an aerosol depends to a great extent on the temperature and air humidity. If with an increase of temperature bacterial decay increases regularly, then with a change of humidity the velocity of decay of various microorganisms changes diversely. De Ome (1944) noted the acceleration of the decay process in Salmonella pullorum during an increase of the relative humidity of the air from 15 to 80%. On the other hand, dry air promoted the destruction of group C streptococci (Wells and Zappasadi, 1942), and the causative agent of pasteurellosis of birds (Yarnykh, 1957). The decay velocity of Bact. prodigiosum in a humidity of 30% was many times higher than at 70% (Griffin and others, 1956; Webb, 1959, 1960). Based on the data of Dunklin and Puck (1948), pneumococci, streptococci, and stapholococci perished most rapidly in a relative humidity of from 45 to 55%. Bact. tularense is most stable at humidities of 25 and 95% and perishes most rapidly at 60%, while P. pestis is most stable in an air humidity of 40% (Beebe and Pirsch, 1958).

The data obtained by us also testifies to the fact that the various microorganisms react diversely to a change of air humidity. A change in the colloidal properties of bacterial drops has relative little significance which is observed mainly at very high and very low levels of humidity in experiments with Staph. albus and Sarcina lutea. As is known, Sarcina lutea is a characteristic representative of the microflora of the air in premises where Staph. albus is also detected fairly often. A significant stability for Staph. albus and Staph. aureus has been demonstrated in a number of works (Wells, W. and Wells, M., 1936; Rechmenskiy, 1951; Kliewe, Wasilewski, 1952; Yaroshenko, 1957; Vershiogora, 1958, 1960, and others).

Having adapted to the conditions of an air medium, the gram-positive cocci are well preserved in the drop phase of a bacterial aerosol, both at low as well as high levels of air humidity. This is attested to by the gradual reduction of the concentration of cocci in the air which is caused mainly by settling. Somewhat of an acceleration in the process of settling and possibly also the decay process of the bacteria are noted during a high air humidity. We weren't able to corroborate the facts of Dunklin and Puck (1948) that in a relative humidity around 50% more staphylococci are destroyed than at higher and lower levels of humidity.

Gram-negative bacteria (Bact. coli, Bact. prodigiosum), not adapted to the conditions of an air medium and moreover isolated from water, decay extremely rapidly in the drop phase of an aerosol at a low air humidity. Thus, at a relative humidity lower than 50%, an overwhelming number of bacteria perish in the course of the first 10 minutes after the creation of the aerosol, whereas these bacteria are sufficiently well preserved at humidities higher than 70%.

Interesting data was obtained by Skrzynska (1949) during the spraying of a culture of Bact. prodigiosum from an airplane. The sprayed bacteria persisted for a long time and in large quantities in clouds, while above and below the clouds they were found for only a short span of time, which also is an indication of the protective action of a high air humidity on the viability of Bact. prodigiosum in an aerosol. American authors succeeded in obtaining a stable strain of Bact. prodigiosum (Serratia marcescens strain ATCC 274) which was widely used for bacterial aerosols and the conduct of a number of experimental works. However, in a later work by Fincher, Kethley and Cown (1957) there appears a report that this strain began to give off variants which are less stable in an air medium than the original strain. It is interesting to note that in the dust-borne phase of an aerosol, Bact. prodigiosum

decays more rapidly at a 15% humidity and at humidities higher than 50% (Dimmick, 1960).

As is known, gram-positive cocci predominate in the air of closed living quarters, gram-positive bacilli are detected less often, and gram-negative bacilli very rarely. In atmospheric air also, the overwhelming number of bacteria constitute gram-positive bacilli and cocci. Based on data, it can be surmised that gram-positive bacilli and cocci are more adapted to survival during changes in the conditions of the air medium than gram negative bacteria.

The process of bacterial decay during the drop phase of a bacterial aerosol may be presented in the following form. During the transition from a liquid to a gaseous medium, there is a disruption in the conditions for the existence of bacterial cells (mechanical breakdown of the suspension, dessication of the external aqueous film and an increase in it of the concentration of salts) which gives rise to the mass destruction of bacteria during the initial period after dispersion. Thus, based on the facts of Ratcliffe (1952) and Wasilewski (1952), 97.5 - 99.2% of tubercular bacilli perish during the establishment of an artificial bacterial aerosol. Subsequently, that is after the drying up of the external aqueous film and the stabilization of the aerosol, the process of bacterial decay slows down, in as much as now it depends on such slowly acting factors as deficiency of nutritive substances, breaking down of the functions of fermentation systems and metabolism, and also the more active processes of oxidation under the conditions of an aerosol. In the opinion of Beebe (1959, 1960), the destruction of bacteria in an aerosol is dependent on a breakdown of the bonds between the protein molecules and the water in the bacterial cells.

The stated facts make it possible to explain such a rapid destruction of unadapted gram-negative bacteria in an air medium at a low humidity by the extremely rapid evaporation of water from the surface of the bacterial drops which is accompanied by a sharp increase in the concentration of sodium chloride (the suspension of bacteria was prepared in a physiological solution) on the surface of the bacterial cells. With an increase in humidity, the rate of evaporation slows down and even stops at a very high humidity. The reduced rate of water evaporation makes the transition of the bacteria from the liquid to a gaseous medium less sharp and makes it possible for these microorganisms to adapt themselves to existence in the conditions of an air medium.

CONCLUSIONS

1. A bacterial drop-phase aerosol was produced in an experimental chamber with a 250 liter capacity. Staph. albus and Sarcina lutea, isolated from the air of a room, and Bact. coli and Bact. prodigiosum, isolated from water, were used as experimental models.

2. A lessening of the concentration of viable microorganisms in the air is a complex process which in a closed space depends on the processes of bacterial decay and on their sedimentation; along with this, the various species of bacteria are characterized by various stability in the conditions of an air medium.

3. The microorganisms Staph. albus and Sarcina lutea, which have adapted to the conditions of an air medium, are well preserved in a relative air humidity from 12 to 98% with small fluctuations of temperature within the limits of 18.5 to 21 degrees. These microorganisms are stable in the drop phase of a bacterial aerosol and are removed mainly due to sedimentation.

4. The microorganisms Bact. coli and Bact. prodigiosum, which are isolated from water and which are not adapted to the conditions of an air medium are considerably less stable in an aerosol. They decay extremely rapidly in a relative air humidity lower than 50%, whereas at a higher humidity they preserve their viability very well. A relative air humidity higher than 70% is most favorable for the survival of Bact. coli and Bact. prodigiosum in a bacterial drop phase aerosol in a temperature between 18.5 and 21 degrees.

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LITERATURE

Vershigora, A. Ye., 1958, Reports of the AN URSR, 9, 1019.

Vershigora, A. Ye., 1960, Methods of Biological Investigations of the Air, Kiev.

Rechmenskiy, S. S., 1951, Problems of Infection by the Aerial Route, M.

Yarnykh, V. S., 1957, Works of the Institute of Veterinary Science, Sanitation, and Ectoparasitology, 12, 196.

Yaroshenko, V. A., 1957, Collection of Scientific Works from the Kiev Institute for the Advancement of Surgeons, p. 212.

Beebe, J. M. and Pirsch, G. W., 1958, Appl. Microbiol., 6, 127.

De Ome, K. W., 1944, Amer. J. Hygiene, 40, No. 3, 239.

Dimmick, R., 1960, J. Bact., 80, No. 3, 289.

Duncline, E. W. and Puck, T. T., 1948, J. Exp. Med., 87, 87.

Fincher, E. L., Kethley, T. W. and Cown, W. B., 1957, Appl. Microbiol., 5, No. 2, 131.

Griffin, C. W., Kantzes, H. L., Ludford, P. M., and Pelczar, M. J., 1956, Appl. Microbiol., 4, No. 1,17.

Kliewe, H. and Wasilewski, E. V., 1952, Z. Hygiene, 134, Nr. 1, 1.

Ratcliffe, H. L., 1952, Amer. J. Hygiene, 55, No. 1, 36.

Skrzynska, J., 1949, Med. doswiadcz. i. mikrobiol., I, 294.

Thomas, J. P., 1955, Dissert. Abstr., 15, No. 9, 1481.

Wasilewski, E. V., 1952, Z. Hygiene, 135, 553.

Webb, S., 1959, Canad. J. Microbiol., 5, N 6, 649.

Webb, S., 1960, Canad. J. Microbiol., 6, N 1, 71, 89.

Wells, W., Wells, M., 1936, J. Amer. Med. Ass., 107, 1698.

Wells, W., and Zappasadi, P., 1942, Science, 96, No. 2490, 277.

Table.

Number of Staph. albus colonies on dishes in samples taken at various times after spraying the suspension.

Relative Air Humidity (%)	Immediately after spraying	After 10 minutes	After 20 minutes
13	4400	2060	800
38	4600	2480	890
56	4200	2200	960
78	4000	1880	860
92	4800	1160	640

Figure 1.

Influence of air humidity on the viability of Bact. coli.

1 - Relative humidity lower than 20%
2 - From 30 - 40%
3 - From 50 - 60%
4 - From 70 - 80%
5 - Higher than 90%

Figure 2.

Influence of air humidity on the viability of Bact. prodigiosum.

1 - Relative humidity lower than 20%
2 - From 30 - 40%
3 - From 50 - 60%
4 - From 60 - 70%
5 - Higher than 90%

Figure 3.

Colonies of Bact. prodigiosum on membrane filters. Colonies of apigmentary variants are visible.

Lower filter - Culture of 5 liters of air at a relative humidity of 37%;

Upper left - at 75%;

Upper right - At an air humidity of 93%.

Explanation of graphs (figures 1 and 2).

A. Number of colonies.

B. Immediately after spraying

C. After 10 minutes.

D. After 20 minutes.

E. Time samples were taken.

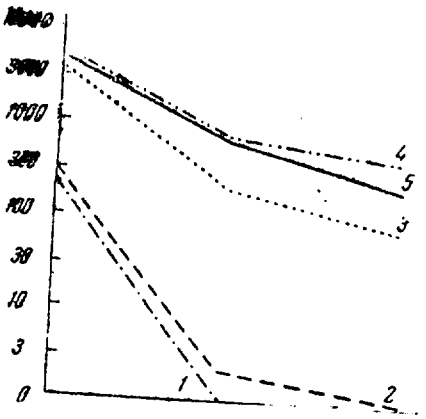

Figure I

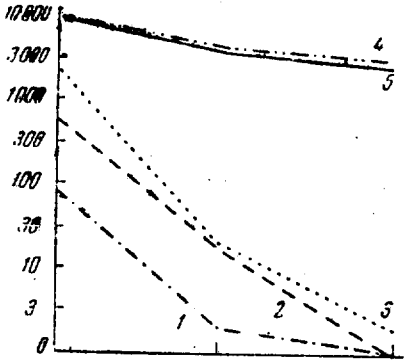

Figure II

Figure III